Biochemical Markers of Bone Turnover and Risk of Incident Diabetes in Older Women: The Cardiovascular Health Study

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OBJECTIVE

To investigate the relationship of osteocalcin (OC), a marker of bone formation, and C-terminal cross-linked telopeptide of type I collagen (CTX), a marker of bone resorption, with incident diabetes in older women.

RESEARCH DESIGN AND METHODS

The analysis included 1,455 female participants from the population-based Cardiovascular Health Study (CHS) (mean [SD] age 74.6 [5.0] years). The cross-sectional association of serum total OC and CTX levels with insulin resistance (HOMA-IR) was examined using multiple linear regression. The longitudinal association of both markers with incident diabetes, defined by follow-up glucose measurements, medications, and ICD-9 codes, was examined using multivariable Cox proportional hazards models.

RESULTS

OC and CTX were strongly correlated (r = 0.80). In cross-sectional analyses, significant or near-significant inverse associations with HOMA-IR were observed for continuous levels of OC (β = −0.12 per SD increment; P = 0.004) and CTX (β = −0.08 per SD; P = 0.051) after full adjustment for demographic, lifestyle, and clinical covariates. During a median follow-up of 11.5 years, 196 cases of incident diabetes occurred. After full adjustment, both biomarkers exhibited inverse associations with incident diabetes (OC: hazard ratio 0.85 per SD [95% CI 0.71–1.02; P = 0.075]; CTX: 0.82 per SD [0.69–0.98; P = 0.031]), associations that were comparable in magnitude and approached or achieved statistical significance.

CONCLUSIONS

In late postmenopausal women, lower OC and CTX levels were associated with similarly increased risks of insulin resistance at baseline and incident diabetes over long-term follow-up. Further research to delineate the mechanisms linking abnormal bone homeostasis and energy metabolism could uncover new approaches for the prevention of these age-related disorders.

Diabetes and osteoporosis are aging-related conditions of which the burden is increasing amid rising life expectancy in developed countries (1,2). Besides having a marked prevalence in older adults, disorders of glucose metabolism and bone homeostasis also are interrelated. Diabetes is well recognized to affect bone health, contributing to decreased bone formation, increased bone marrow adiposity, and a

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heightened risk of fracture (3,4). Aging-related inflammation and oxidative stress also promote the development of both osteoporosis, mainly by reducing bone formation (5), and diabetes (6). Furthermore, insofar that skeletal integrity both determines and is determined by physical activity (7), bone itself may influence diabetes risk. Evidence from animal models, however, suggests that the influence of bone remodeling on glucose dysregulation is more direct, implicating bone-derived factors as modulators of whole-body energy metabolism (8).

Osteocalcin (OC), a protein secreted by osteoblasts, serves as a marker of bone formation (9). Apart from its associations with bone remodeling and fractures, OC has been shown to be a metabolically active hormone in animal models, promoting pancreatic β-cell mass and insulin secretion while also enhancing insulin sensitivity in skeletal muscle and adipose tissue (10,11). OC undergoes γ-carboxylation at glutamate residues, which leads to retention in bone through avid binding to hydroxyapatite; its release of the undercarboxylated form (ucOC) into the circulation accounts for the peptide's hormonal actions (9). Because bioactive ucOC is difficult to measure, however, clinical investigation has largely focused on total OC. In cross-sectional studies in humans, total OC has been documented to be inversely associated with plasma glucose, fat mass, insulin resistance, and metabolic syndrome severity (12). Few longitudinal studies, however, have evaluated the relationship between total OC and incidence of diabetes. One such study documented a significant inverse association (13), but a second failed to replicate this finding (14).

C-terminal cross-linked telopeptide of type I collagen (CTX), a marker of bone resorption and osteoclast activity, recently has been linked to the development of dysglycemia in a small clinical study (15). Mouse models suggest that bone resorption is a necessary prerequisite for the decarboxylation of OC, leading to its hormonally active form (11). The simultaneous assessment of a marker of bone resorption along with OC may shed light on the contribution of osteoclast function to the role of OC in the development of diabetes as well as inform the pathobiology of bone and energy metabolism in humans. In view of the sparse existing data on the prospective association of two major biochemical markers of bone turnover with diabetes, we addressed this question in a well-characterized U.S. cohort of older adults with long-term follow-up, focusing on postmenopausal women, the group at greatest risk of metabolic bone disease. Our primary hypothesis was that OC would be inversely related to diabetes, with CTX exhibiting a similar association secondarily through the well-known linkage between bone formation and resorption.

RESEARCH DESIGN AND METHODS

The Cardiovascular Health Study (CHS) is a prospective, population-based cohort study in community-dwelling ambulatory adults ages ≥65 years. As previously detailed (16), eligible participants were sampled from Medicare eligibility lists at four field centers in the U.S. (California, Maryland, North Carolina, and Pennsylvania). An original cohort of 5,201 mostly white participants was recruited in 1989–1990, followed by a supplemental cohort of 697 predominantly African American participants in 1992–1993. Participants underwent standardized health assessments for demographic data, lifestyle habits, medical history, medications, and laboratory and diagnostic testing, as detailed elsewhere (17,18). The CHS coordinating center and all field centers received institutional review board approval for the study, and participants gave informed consent. The current study was approved by the institutional review board at Albert Einstein College of Medicine.

For this study, we included 1,760 female participants who completed the 1992–1993 examination, had never-thawed fasting serum specimens available that were collected during this visit, and underwent OC and CTX measurement as part of an ancillary study evaluating the relationships of bone turnover markers with metabolic, skeletal, and cardiovascular outcomes. Of these participants, we excluded 248 with prevalent diabetes, 5 whose diabetes status was unknown, and 52 who were taking either oral glucocorticoids or vitamin K antagonists, resulting in a final sample of 1,455 participants.

Serum specimens typically were collected during a morning visit after an 8-h overnight fast. Specimens were stored at −80°C until measurement, which was performed in 2017 at the Laboratory for Clinical Biochemistry Research at the University of Vermont. Total OC was measured using electrochemiluminescent immunoanalysis (Roche Diagnostics, Indianapolis, IN), an approach that quantifies both the intact peptide and its largest proteolytic fragment, the N-terminal midmolecule. The interassay coefficient of variation range was 2.1–3.7%. CTX was measured using a Roche β-CrossLaps assay (Roche Diagnostics). The interassay coefficient of variation range was 6.3–11.5%.

Ascertainment of diabetes was based on biennial blood glucose measurements from 1992–1993 through 1998–1999, harmonized as previously described (19), and by annual medication inventory supplemented by Centers for Medicare & Medicaid Services claims. Diabetes was defined as a fasting blood glucose of >125 mg/dL, a nonfasting glucose >199 mg/dL, use of antiglycemic medication, or inpatient or outpatient ICD-9 codes (20).

All covariates included in this study were collected at the 1992–1993 examination. HOMA to estimate insulin resistance (HOMA-IR) was calculated as [fasting glucose (mmol/L) × fasting insulin (mU/L)]/22.5 (21). Estimated glomerular filtration rate (eGFR) was calculated on the basis of serum creatinine concentration with the Chronic Kidney Disease Epidemiology Collaboration equation (22). Physical activity was measured in kilocalories per week using previously published methods (17). Definitions and methods of ascertainment of prevalent cardiovascular disease (CVD) (coronary heart disease [CHD], stroke, congestive heart failure [CHF], and atrial fibrillation [AF]) have been reported previously (23,24). Prevalent hip fractures were identified using ICD-9 codes from hospitalization records that were prospectively collected in the CHS. Hip fracture was defined as ICD-9 code 820.xx.

Distributions of OC and CTX levels were assessed by visual inspection of histograms (Fig 1). Their correlation was assessed by the Pearson method. Cross-sectional associations of bone turnover markers with HOMA-IR were evaluated using multiple linear regression. Confounders were selected for

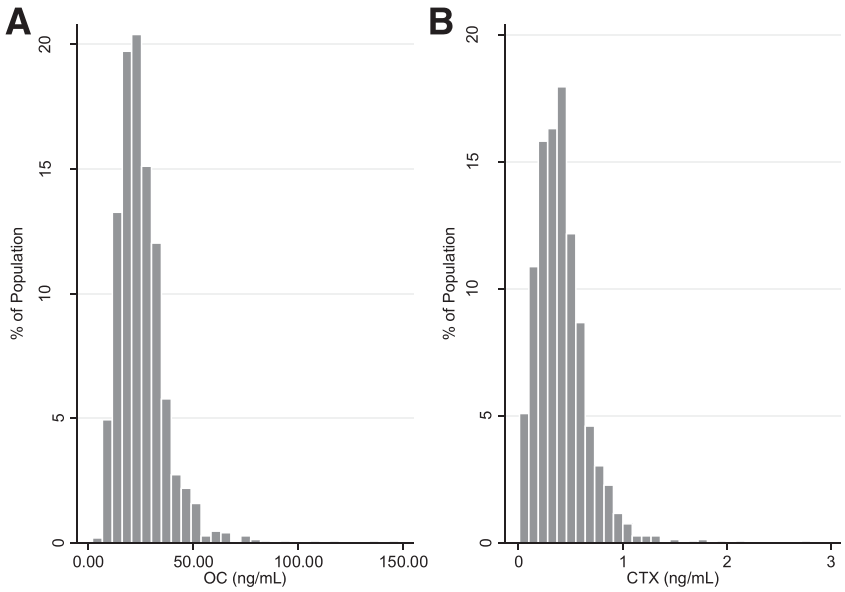

Figure 1—Distribution of OC (*A*) and CTX (*B*) in the study sample.

adjustment in sequential models on the basis of prior associations or known biological mechanisms. Initial adjustment was performed for age, race, and field center. In the fully adjusted model, we also included BMI, systolic blood pressure, treatment with antihypertensive medications, smoking status, alcohol consumption, physical activity, hormone replacement therapy (HRT), LDL, prevalent CHD, prevalent CHF, prevalent stroke, prevalent AF, and eGFR. Variables related to insulin resistance, including HDL and triglycerides, and therefore in the causal pathway were not included. The functional form of the relationship between each biomarker and the primary outcome was evaluated using generalized additive models. No evidence of meaningful nonlinearity was found; therefore, each biomarker was modeled continuously (per SD increment) in Cox proportional hazard models while adjusting for the same potential confounders as above. Adjusted associations with diabetes for quartiles of each biomarker also were examined in secondary analyses. The proportionality assumption was tested using Schoenfeld residuals. In separate sensitivity analyses, we excluded participants taking vitamin D supplements (n = 2), activated vitamin D (calcitriol) (n = 2), and bisphosphonate medications (n = 10 in addition to one participant taking vitamin D and a bisphosphonate); those taking HRT (n = 214); those taking thiazide diuretics (n = 279); those with a history of hip fracture (n = 14); and those with prevalent CHD, CHF, stroke, and AF (n = 312). In addition, we restricted the sample to participants with available 25-hydroxyvitamin D (25-OHD) measurements (n = 1,101) and adjusted for these 25-OHD levels.

All analyses were performed with Stata 14.2 statistical software (StataCorp, College Station, TX). A two-tailed $P < 0.05$ defined statistical significance.

RESULTS

The mean ± SD age of the study sample was 74.6 ± 5.0 years, and 12.9% of participants were African American. A strong correlation was found between serum concentrations of OC and CTX (r = 0.80; $P < 0.001$). The distributions of baseline covariates by quartiles of circulating bone turnover marker levels are shown in Table 1. Serum OC concentrations were positively associated with age, systolic blood pressure, LDL, and prevalence of CHD and CHF; serum OC levels were inversely associated with BMI, HOMA-IR, HDL, triglycerides, C-reactive protein (CRP), eGFR, and HRT. Serum CTX levels were positively associated with age and systolic blood pressure and inversely associated with BMI, HOMA-IR, HDL, triglycerides, HRT, and calcium supplementation.

After adjustment for demographic, lifestyle, clinical, and laboratory covariates, continuous levels of OC were significantly inversely related to HOMA-IR (β = −0.12 per SD increment; P = 0.004). A similar inverse relationship was found for continuous CTX levels with HOMA-IR, although this was marginally nonsignificant (β = −0.08 per SD increment; P = 0.051).

During a median follow-up of 11.5 years (interquartile range 6.8–16.9 years, maximum 17.6 years), 196 participants developed diabetes (12.1 per 1,000 person-years). Table 2 shows the unadjusted incidence of diabetes by quartiles of OC and CTX. The lowest rates of diabetes were observed for the highest quartiles of serum OC and CTX, with a more graded relationship across increasing quartiles apparent for CTX in these unadjusted analyses.

Table 2 also presents the adjusted associations of serum OC and CTX with incident diabetes. Generalized additive model plots showed no apparent departure from linearity for either biomarker in relation to diabetes; therefore, hazard ratios (HRs) for continuous serum levels of the biochemical markers are presented, scaled per SD increment of the respective biomarker. After full adjustment, there were comparable inverse associations with incident diabetes for continuous levels of both OC (HR 0.85 per SD increment [95% CI 0.71–1.02]; P = 0.075) and CTX (0.82 [0.69–0.98]; P = 0.031) that approached or achieved statistical significance. Additional adjustment for CRP did not meaningfully change these estimates. Secondary examination of quartiles of OC and CTX did not reveal significant associations with incident diabetes.

Figure 2 shows the adjusted relationships of the two biomarkers with diabetes after separately excluding women taking vitamin D supplements, activated vitamin D (calcitriol), or bisphosphonates; those taking HRT or thiazide diuretics; those with a history of hip fracture or prevalent CVD; and those with available 25-OHD levels before and after adjustment for such levels. These sensitivity analyses did not show any meaningful differences in the risk estimates of incident diabetes for OC or CTX.

CONCLUSIONS

We investigated the associations of two biochemical markers of bone turnover with incident diabetes in older women from a population-based study. The analyses of OC, a marker of bone formation experimentally linked to energy metabolism, and CTX, a marker of bone

Table 1—Baseline characteristics of study sample by quartiles of bone turnover markers

	Quartile							
	OC				CTX			
Characteristic	Q1 (*n* = 364)	Q2 (*n* = 364)	Q3 (*n* = 364)	Q4 (*n* = 363)	Q1 (*n* = 364)	Q2 (*n* = 364)	Q3 (*n* = 364)	Q4 (*n* = 363)
Bone turnover marker range (ng/mL)	≤17.71	17.72–23.61	23.62–30.84	>30.84	≤0.25	0.26–0.38	0.39–0.52	>0.52
Age (years)	73.7 ± 4.6	74.2 ± 4.7	74.4 ± 5.0	76.1 ± 5.5	73.3 ± 4.3	74.7 ± 5.0	74.4 ± 4.8	75.9 ± 5.6
African American, *n* (%)	56 (15.4)	51 (14.0)	56 (15.4)	25 (6.9)	48 (13.2)	48 (13.2)	49 (13.5)	43 (11.8)
BMI (kg/m^2)	30.0 ± 4.8	30.0 ± 4.9	26.3 ± 5.1	25.2 ± 4.6	26.8 ± 4.6	26.7 ± 4.9	26.5 ± 5.3	25.3 ± 4.7
Education, *n* (%)								
< High school	92 (25.3)	98 (27.0)	100 (27.5)	99 (27.3)	87 (24.0)	101 (27.8)	83 (22.8)	118 (32.6)
High school graduate	114 (31.3)	113 (31.1)	106 (29.2)	101 (27.9)	114 (31.4)	107 (29.5)	121 (33.2)	92 (25.4)
> High school	158 (43.4)	152 (41.9)	157 (43.3)	162 (44.8)	162 (44.6)	155 (42.7)	160 (44.0)	152 (42.0)
Smoking status, *n* (%)								
Never smoked	180 (49.5)	211 (58.0)	221 (60.7)	228 (62.8)	186 (51.1)	219 (60.2)	217 (59.6)	218 (60.1)
Former smoker	140 (38.5)	124 (34.1)	108 (29.7)	103 (28.4)	139 (38.2)	114 (31.3)	113 (31.0)	109 (30.0)
Current smoker	44 (12.1)	29 (8.0)	35 (9.6)	32 (8.8)	39 (10.7)	31 (8.5)	34 (9.3)	36 (9.9)
Alcoholic beverages/week	1.8 ± 4.8	1.4 ± 4.0	1.1 ± 3.5	1.1 ± 3.0	1.6 ± 4.2	1.4 ± 4.2	1.2 ± 3.5	1.2 ± 3.7
Total physical activity (kcal/week)	1,370 ± 1,644	1,299 ± 1,456	1,269 ± 1,478	1,273 ± 1,761	1,389 ± 1,666	1,281 ± 1,435	1,304 ± 1,563	1,237 ± 1,681
SBP (mmHg)	134.7 ± 21.2	135.7 ± 21.8	136.8 ± 23.2	137.8 ± 22.3	134.3 ± 20.6	136.3 ± 22.1	136.2 ± 22.3	138.2 ± 23.5
Antihypertensive therapy, *n* (%)	176 (48.4)	191 (52.5)	154 (42.3)	173 (47.7)	179 (49.2)	183 (50.3)	149 (40.9)	183 (50.4)
Thiazide diuretic, *n* (%)	95 (26.1)	79 (21.7)	50 (13.7)	55 (15.2)	99 (27.2)	74 (20.3)	61 (16.8)	45 (12.4)
HRT, *n* (%)	127 (34.9)	39 (10.7)	34 (9.3)	14 (3.9)	141 (38.7)	41 (11.3)	23 (6.3)	9 (2.5)
Glucose (mmol/L)	5.4 ± 0.5	5.4 ± 0.6	5.3 ± 0.5	5.3 ± 0.5	5.4 ± 0.6	5.4 ± 0.5	5.4 ± 0.6	5.3 ± 0.5
Insulin (IU/mL)	11.7 ± 8.0	11.2 ± 5.9	10.5 ± 5.4	10.0 ± 4.6	11.5 ± 7.6	10.9 ± 5.8	10.8 ± 6.1	10.2 ± 4.5
HOMA-IR	2.8 ± 2.1	2.7 ± 1.6	2.5 ± 1.4	2.4 ± 1.12	2.8 ± 2.1	2.6 ± 1.6	2.6 ± 1.6	2.4 ± 1.1
LDL (mmol/L)	6.4 ± 1.8	7.1 ± 1.9	7.0 ± 1.9	7.1 ± 1.8	6.6 ± 1.9	7.0 ± 1.8	7.1 ± 1.8	6.9 ± 2.0
HDL (mmol/L)	3.5 ± 0.9	3.2 ± 0.8	3.2 ± 0.8	3.2 ± 0.7	3.5 ± 0.9	3.3 ± 0.8	3.2 ± 0.8	3.1 ± 0.7
25-OHD (ng/mL)†	28.3 ± 17.2	24.4 ± 9.0	23.3 ± 9.0	23.0 ± 9.3	28.1 ± 17.1	24.6 ± 9.7	22.9 ± 9.2	23.5 ± 8.6
Triglycerides (mmol/L)	8.6 ± 5.1	7.7 ± 4.0	7.7 ± 4.6	7.6 ± 3.5	8.8 ± 5.3	8.0 ± 4.6	7.5 ± 3.9	7.3 ± 3.3
CRP (mmol/L)	0.3 ± 0.4	0.3 ± 0.5	0.2 ± 0.5	0.2 ± 0.3	0.3 ± 0.4	0.3 ± 0.4	0.3 ± 0.5	0.2 ± 0.5
eGFR (mL/min/1.73 m^2)	74.7 ± 14.5	73.3 ± 14.3	71.8 ± 15.8	68.0 ± 18.0	74.5 ± 14.2	71.4 ± 15.1	72.5 ± 15.9	69.3 ± 17.9
Prevalent CHD, *n* (%)	46 (12.6)	67 (18.4)	62 (17.0)	80 (22.0)	50 (13.7)	62 (17.0)	56 (15.4)	87 (24.0)
Prevalent CHF, *n* (%)	8 (2.2)	9 (2.5)	13 (3.6)	23 (6.3)	7 (1.9)	13 (3.6)	14 (3.8)	19 (5.2)
Prevalent stroke, *n* (%)	8 (2.2)	14 (3.8)	12 (3.3)	13 (3.6)	7 (1.9)	14 (3.8)	12 (3.3)	14 (3.9)
Prevalent AF, *n* (%)	4 (1.1)	5 (1.4)	4 (1.1)	7 (1.9)	6 (1.6)	5 (1.4)	5 (1.4)	4 (1.1)

Data are mean ± SD for continuous variables. SBP, systolic blood pressure. †Missing in *n* = 354.

Table 2—Unadjusted incidence and HRs of diabetes by quartiles and per SD of serum OC and CTX

	Person-years	Incident diabetes, *n*	Unadjusted incidence/1,000 person-years (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
OC (ng/mL)					
≤17.71 (Q1)	4,327	50	11.56 (8.76–15.25)	1.00 (Ref.)	1.00 (Ref.)
>17.71–23.61 (Q2)	4,050	56	13.83 (10.64–17.97)	1.20 (0.82–1.76)	1.13 (0.76–1.68)
>23.61–30.84 (Q3)	4,076	54	13.25 (10.15–17.30)	1.15 (0.78–1.69)	1.11 (0.74–1.67)
>30.84 (Q4)	3,763	36	9.56 (6.90–13.26)	0.85 (0.55–1.31)	0.83 (0.52–1.31)
Per SD increase				0.88 (0.74–1.04)	0.85 (0.71–1.02)
CTX (ng/mL)					
≤0.25 (Q1)	4,218	58	13.75 (10.63–17.70)	1.00 (Ref.)	1.00 (Ref.)
>0.25–0.38 (Q2)	4,178	52	12.44 (9.48–16.33)	1.20 (0.82–1.76)	1.13 (0.76–1.68)
>0.38–0.52 (Q3)	4,023	49	12.18 (9.21–16.12)	1.15 (0.78–1.69)	1.11 (0.74–1.67)
>0.52 (Q4)	3,795	37	9.75 (7.07–13.46)	0.85 (0.55–1.31)	0.83 (0.52–1.31)
Per SD increase				0.85 (0.72–1.00)	0.82 (0.69–0.98)

resorption, revealed consistent associations in magnitude and direction of these two biomarkers with dysglycemic outcomes. Lower levels of serum OC and CTX were associated cross-sectionally with higher HOMA-IR and longitudinally with an increased risk of diabetes, associations that approached or achieved statistical significance for both outcomes. Furthermore, sensitivity analyses showed that the association of either biomarker with the primary outcome of incident diabetes was not meaningfully altered by exclusion of participants taking vitamin D supplements and bisphosphonates, HRT, or thiazide diuretics; with a history of hip fracture; and with prevalent CVD. Adjustment for 25-OHD levels in participants with available measurements also did not materially influence the results.

Taken together, the findings show that low levels of these two strongly correlated markers, indicating low bone turnover, are associated with a higher incidence of diabetes. Contrary to our primary hypothesis, serum total OC, a measure of bioactive circulating ucOC, albeit an imperfect one (11), did not emerge as the dominant biomarker for this relationship. However, the results for OC are broadly consistent with those from a meta-analysis that examined the association of this biomarker with incident diabetes (12). The meta-analysis reported a pooled relative risk of 0.89 (95% CI 0.78–1.01) for the comparison of extreme quartiles of OC across cohort studies along with evidence of significant inverse associations with prevalent diabetes, serum glucose levels, and insulin resistance. By contrast, the current findings for CTX differ from the results of the sole longitudinal investigation of serum CTX in relation to the combined 4-year incidence of prediabetes and diabetes, which described the highest rates for the top and bottom tertiles of CTX, with a significant positive adjusted association for the top compared with the middle tertile (15). This investigation, however, was performed in a small referral-based sample of pre- and postmenopausal middle-aged Chinese women, with a modest number of outcomes consisting mostly of prediabetes.

The present findings among late-postmenopausal women suggest that decreased bone remodeling and the factors underlying it may be more relevant determinants of dysregulated glucose metabolism in this population than the possible direct effects attributable to OC. In older women, aging-related inflammation and oxidative stress, rather than estrogen deficiency, may be the major contributors to the imbalance between bone formation and resorption (25). Such pathophysiology differs from that relevant to middle-aged women, in whom estrogen depletion, and the resulting increase in bone resorption, could underlie the link between higher CTX levels and glycemic dysregulation (7). In late-postmenopausal women, heightened inflammation also impairs insulin-sensitive tissues and pancreatic β-cell secretory capacity (26). Such parallel influences of a proinflammatory or prooxidant state on bone, adipose tissue, skeletal muscle, and pancreas could contribute to the relationship observed between reduced bone turnover and new-onset diabetes. Of note, the observed associations persisted after adjustment for CRP, but such adjustment may not have adequately captured local oxidative stress across these tissues.

The current findings may be better understood in the context of preclinical insights gained from mouse models. Convincing pathobiological studies in mice have led to the identification of OC as a hormone that promotes not only pancreatic β-cell proliferation and insulin secretion but also decreased fat mass, higher adiponectin secretion, and increased energy expenditure along with enhanced insulin sensitivity (11). However, OC is primarily secreted in a γ-carboxylated form, allowing it to integrate into the bone extracellular matrix by increasing its affinity for hydroxyapatite. To attain its endocrine function, decarboxylation is necessary because ucOC is the hormonally active form (27). Available evidence suggests an important role for osteoclasts in post-translationally modulating the decarboxylation of OC and regulating glucose homeostasis in mice (28). Osteoclast depletion in mice causes a reduction in serum ucOC levels and impairs glucose tolerance. Prevailing theory suggests that bone resorption (reflected by CTX levels) and the acidic environment of the resorption lacunae created by osteoclasts are needed for the decarboxylation of OC.

Given this experimental evidence, the observed link between reduced bone remodeling and the heightened risk of diabetes observed herein could result from diminished secretion of carboxylated OC stemming from reduced bone formation (29). The observed association also could be a consequence of decreased bone resorption, with which bone formation is closely linked (30), and diminished

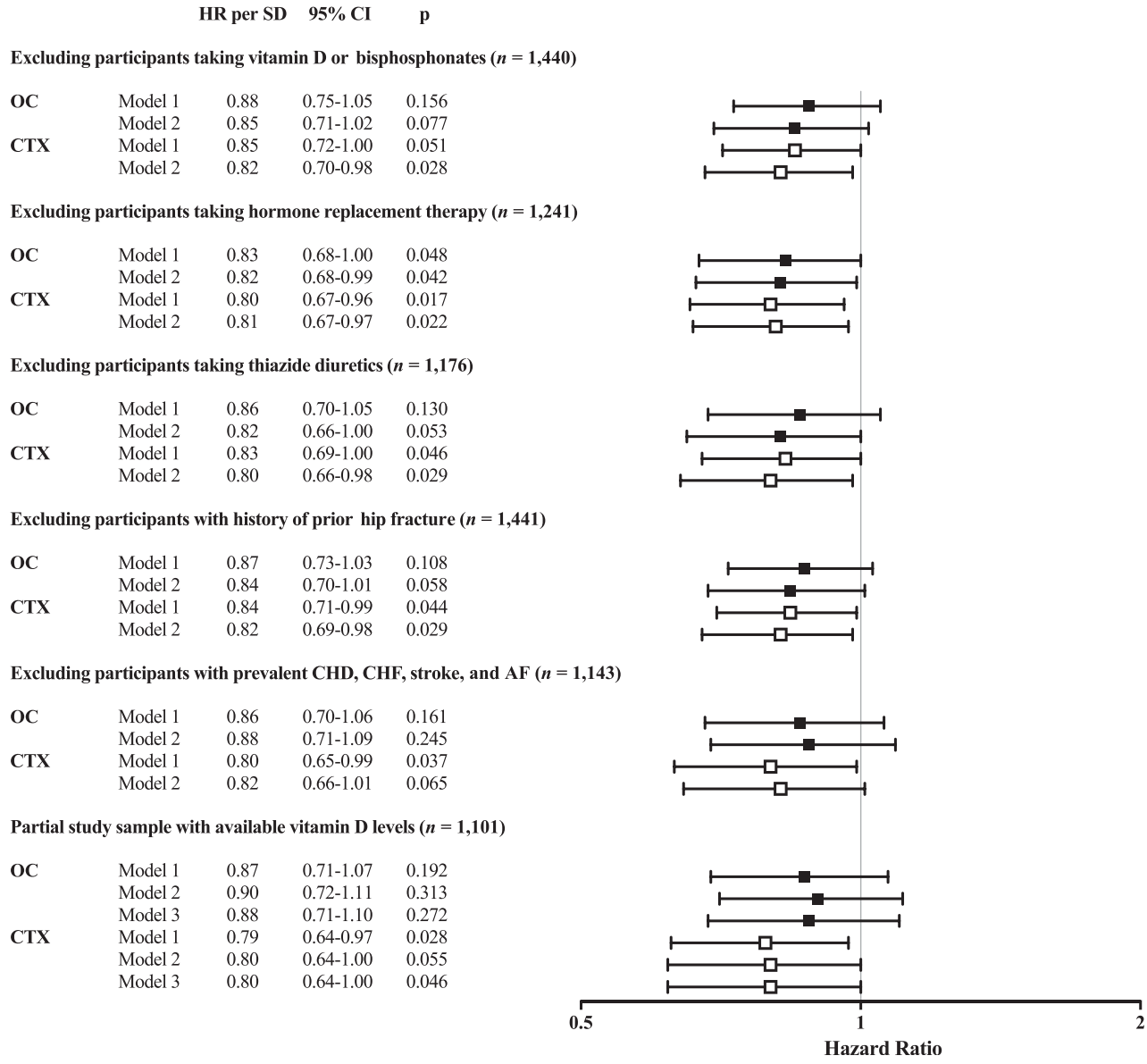

Figure 2—Sensitivity analyses examining the associations of serum OC and CTX with incident diabetes in selected subgroups of the study sample. Model 1 is adjusted for age, race, and field center. Model 2 is additionally adjusted for BMI; systolic blood pressure; antihypertensive therapy; smoking status; alcohol consumption; physical activity; HRT; prevalent CHD, CHF, stroke, and AF; LDL; and eGFR. Model 3 is adjusted for 25-OHD levels in participants with available data.

generation of active ucOC. In this regard, our measurement of total OC, but not ucOC, may have led to an underestimation of the association of OC with the primary outcome. Direct assessment of ucOC, however, is unreliable because the decarboxylation on the single Glu13 residue caused by the low pH in the resorption lacunae is not readily measured by available assays, which measure ucOC more generally (11). To wit, available studies that have measured both total OC and ucOC have found similar cross-sectional associations with prevalent diabetes for both measures (31). Hence, although the high correlation between total OC and CTX, and indeed between bone formation and resorption, precludes separating their contributions to the association with diabetes documented here, future efforts at improving quantitation of ucOC could yield further insights into the biology potentially underlying it.

Another important consideration in interpreting the current findings is that glucose regulation itself also appears to exert direct influences on bone metabolism. Studies in mice have shown that insulin stimulates osteoblast development and OC expression (32). Furthermore, insulin signaling suppresses osteoprotegerin production, resulting in increased bone resorption and release of ucOC into the bloodstream (33). These and other experimental findings in mouse models and human cell culture support the concept that normal insulin signaling in bone leads to a feed-forward loop in OC activation (33). By contrast, a cross-sectional clinical study in middle-aged adults showed that hyperinsulinemia during the hyperinsulinemic-euglycemic clamp suppressed both OC and CTX levels, more so in insulin-sensitive than in insulin-resistant participants or those with diabetes whose levels were already low (34). This finding led to the proposition that hyperinsulinemia may instead

suppress production of OC in humans, even in the setting of insulin resistance in muscle and fat, through unaffected insulin responsive pathways at the level of the osteoblast (34). These discrepancies between experimental and clinical studies remain to be explained but could owe to interspecies differences in OC and insulin signaling or other factors.

Regardless, prevalent insulin resistance and associated hyperinsulinemia, whether through affected or spared insulin responsive pathways in osteoblasts, could account for the decreased bone remodeling observed in the current study being linked to incident diabetes. Moreover, hyperglycemia itself has been shown experimentally to inhibit osteoclast development (35), and formation of advanced glycation end products can stimulate osteoblast apoptosis (36). We removed participants with prevalent diabetes, but the possibility that unrecognized diabetes may have affected bone remodeling at baseline cannot be excluded.

Of additional relevance to the current findings are clinical data on antiresorptive agents, medications that lead to reductions in levels of bone turnover markers (37). In secondary analyses of randomized trials, these agents did not show a meaningful effect on measures of glycemia or diabetes risk (37). These data call into question whether experimental findings from mouse models on the metabolic actions of OC apply to humans. The results, however, come from postmenopausal women selected for osteoporosis who had a lower BMI and lower risk of diabetes than the general U.S. population. Moreover, the follow-up periods in question were ~3–4 years, a shorter time horizon than that of the current study. Such lower susceptibility and lack of long-term follow-up may have masked development of hyperglycemia in these analyses. Hence, whether the findings of the current study reflect true bioactive consequences of OC deficiency, parallel actions of oxidative stress in bone and adipose tissue/pancreas, reverse causality from baseline insulin resistance, or a combination of all three will require further investigation.

This study has several strengths. CHS is a well-characterized cohort with long-running follow-up. Laboratory analysis of bone turnover markers for this ancillary study was performed on never-thawed serum specimens stored at −80°C after collection under standardized conditions, thereby keeping biomarker degradation to a minimum. Participants with prevalent and incident diabetes were assessed through a combination of laboratory methods, medication inventory, and ICD-9 codes, enhancing ascertainment. In addition, we simultaneously measured a marker of bone formation with one of bone resorption to capture both components of bone turnover and obtain a more complete picture than is possible by determining only one facet of bone metabolism.

Several limitations also must be considered. First, the study focused on predominantly white women in late postmenopause, so the findings do not necessarily apply to men, younger women, or other ethnic groups. Second, bone turnover markers capture only a snapshot in time of bone mineral metabolism, a dynamic process, and may be influenced by short-term events that occurred prior to venipuncture and reflect other processes than the status of chronic bone health, such as unrecognized medications, trauma, or biochemical cross-reactivity. Such factors would tend to bias the associations under study toward the null, however, suggesting that the relationships may be stronger than documented here. Third, measures that potentially could have provided additional information on both glucose metabolism (hemoglobin A_{1c}) and bone metabolism (serum calcium and phosphate) were available only in subsets of the cohort and therefore could not be meaningfully incorporated into the analysis. Fourth, we were able to ascertain and account for prevalent hip fractures using hospitalization ICD-9 codes in the analysis, but lack of outpatient ICD-9 codes prevented the evaluation of other kinds of fractures at baseline. Finally, as noted above, measurement of total OC may not capture all the nuances of OC biology. Still, although total OC can be reliably measured with automated immunoassays, ucOC levels are less reliably quantifiable and standardized (38) and have not been found to be associated with incident diabetes in two of three prospective studies (39–41).

In conclusion, in this cohort of older women, we provide, to our knowledge, the first prospective evidence for an association between reduced bone remodeling and increased risk of incident diabetes. These findings suggest that bone health may play a role in the maintenance of glucose regulation late in life and provide impetus for further investigation of the mechanisms linking bone homeostasis with energy metabolism, which could uncover new preventive approaches to these common comorbidities in our aging population.

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